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Temperature-sensitive periods and autonomy of pleiotropic effects of dl^{ts}, a conditional discless lethal mutation in *Drosophila virilis*

Vladimir G. MITROFANOV[1], XUE Dong[2*], YANG Chang-Ju[2], YAO Ying-Juan[2], CAI Wan-Lun[2]

(1. Institute of Developmental Biology, Russian Academy of Sciences, Moscow 117334, Russia;
2. The Institute of Urban Pest Control, Huazhong Agriculture University, Wuhan 430070, China)

Abstract: A series of temperature-shift experiments have been conducted with homo- and/or hemizygous strains for temperature-sensitive (*ts*) mutations of the discless, dl^{ts} in *Drosophila virilis* Sturt, where the restrictive temperature is 31℃, and the permissive temperature is 25℃. The temperature threshold is set at 29℃ and fluctuates between 25℃ and 31℃ at an interval of 2℃. The temperature-sensitive period (TSP) is determined by the analysis of both shift-up and shift-down experiments. Discrete TSPs for lethality are localized on the first, the second and the third larval instar for a 10 hr period after pupation with an interval of a few hours between TSP and the lethal phase (LP). TSPs for adult morphology defects are localized on the second, the third larval instars and the early pupal period. Animals during TSPs are subjected to 12-hr-pulse, 24-hr-pulse, and 48-hr-pulse treatments respectively, and have produced a large number of defects. The defects include: eye scar, clumping pigment eye, small and lozenge-shaped eye, missing facets, small wing, thick wing vein, shortened tarsal segments, fused leg segments, extra macrochaetae or bristles, missing antennal segments, and duplicating palpus and/or lacinia, with distinct patterns of eye, wing, bristle and leg defects observed within the third larval instar. Homoeotic mutants also occur in this period. Each defect has its own specific time-dependent pattern. The patterns of damage are observed in imaginal tissue and all the defects are categorized into three classes: repeat, deletion and repeat and deletion happened at the same time. There is evidence from genetic somatics that there are some specific time patterns for dl^{ts} to produce the pleiotropic effects.

Key words: *Drosophila virilis*; imaginal disc; temperature-sensitive period; lethal phase; pattern formation

Temperature-sensitive (*ts*) mutations are one kind of conditional mutations and have been known for about sixty years (Foster and Suzuki, 1970). The usefulness of *ts* mutations for analysis of a number of different kinds of problems in cell biology has been suggested, and the use of *ts* mutations amenable to genetic, cytological and biochemical techniques of analysis should offer great insights into the molecular biology of eukaryotic organisms (Suzuki, 1971; Vyse and James, 1972; Foster, 1973).

A powerful tool for analyzing certain developmental processes such as pattern formation consists of manipulating the regions of cell death in developing imaginal discs and analyzing the types and frequencies of the structural abnormalities produced in the adult. One technique is temperature-sensitive autonomous cell-lethal mutations. Imaginal disc is one of the most important experimental systems for the study of pattern formation (Bryant, 1978; Martin, 1982; Zecca and Steuhl, 2002a, 2002b; Hipfner and Cohen, 2003; Delanoue *et al*., 2004).

One of the attractive features of *Drosophila* for the study of pattern formation during the development is the extreme intricacy and uniformity of the patterns of hairs, bristles and sense organs. Furthermore, the widespread covering of hairs and bristles which can be affected by particular mutations offers an ideal situation for the clonal analysis of growth and development. Scanning electron microscopy (SEM) can prove to be very useful in pattern formation study. Scanning electron microscope provides high resolution, large depth of focus and apparent oblique illumination which gives the impression of three-dimensionality, and these qualities often more than compensate for the inability to record the color of the structures (French *et al*., 1976; Girton and Russell, 1981). However, the ultimate objective is to understand the molecular mechanism of imaginal disc development by using specific mutants (Poodry *et al*., 1973; Camposortega and Gatefe, 1976; Ingham, 1985; Breen and Harte, 1991).

At present, many reports about *ts* defects produced by *l(1)N1*^{ts}, a *ts* recessive lethal mutation of the Notch locus in *Drosophila melanogaster* are

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* **通讯作者** Author for correspondence, 薛东,男,1946 年生,副教授,主要从事城市昆虫学研究, E-mail: xuedj@mail.hzau.edu.cn
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available. Mitrofanov (1976) had reported phenogenetics of a temperature-sensitive mutation discless (dl^{ts}) affecting the development of imaginal discs in *Drosophila virilis* Sturt. No further report on it since then. For this reason and to serve the above mentioned two objectives, the aim of the paper is to study the growth of *D. virilis* under different temperatures, and the report describes temperature-shift analyses of flies homo- and hemizygous for dl^{ts} and studies of the expression of dl^{ts} in genetic mosaics. Scanning electron microscopy technique is also used for monitoring adult morphology defects. In short, these studies include: temperature sensitivity studies, detailed description of the abnormal pattern, comparisons with the fate map of the discs, analysis using morphogenetic mosaics, regeneration studies in the mutant and histological studies to detect cell death. In addition to analytical uses, these studies offer a significant practical advantage which will become increasingly important as biochemical and molecular analysis of imaginal disc development proceeds with dl^{ts} in *D. virilis* (Ikeda and Kaplan, 1970; Postlethwalt and Schneiderman, 1973; Leveson and Housman, 1981; Jurgens, 1985).

1 MATERIALS AND METHODS

1.1 Genetic strains and culture conditions

dl^{ts} is a *ts* recessive temperature-sensitive mutation discless (localized on the second chromosome) which arose spontaneously in the Tashkent wild-type line in 1972.

b, *gp*, *cd*, *pe* is a strain marked with these recessive genes, broken (*b*, 2), gapped (*gp*, 3), cardinal (*cd*, 4), peach (*pe*, 5). Here they are used as tester strain. This strain was from the National Drosophila Species Resource Center and now is maintained in Genetic Laboratory of Institute of Developmental Biology, Russian Academy of Sciences, Moscow.

D. virilis were from the Institute of Developmental Biology, Russian Academy of Sciences, Moscow. The strains of *D. virilis* were maintained on a standard-cornmeal-yeast-dextrose food at 25℃ (in the fly room, over 70% humidity). Autosomes and their source were not controlled.

1.2 Temperature-shift studies

A stock of dl^{ts} is maintained at 25℃ and its progeny is used for all shift studies except where noted. Temperature shift-up and shift-down, and pulse treatments are employed during larval and pupal periods separately; each treatment is characterized by the developmental age: by maintaining the temperature at 25℃ (permissive temperature) before increasing it to 31℃ and by maintaining the temperature at 31℃ (restrictive temperature) prior to the shift-down. For temperature shifts in the larval period and pupal period, follow the description of Shellenbarger (1977). Larval molts are timed according to the morphology of mouth parts and anterior spiracles.

For each mutant, the duration of the temperature sensitive (TSP) and the timing of the effective lethal phase (LP) are determined through the analysis of reciprocal shift-up and shift-down experiments. For lethality, the earliest shift-down, for which decreased viability is observed, is identified as the start of the TSP, while the earliest shift-up that permits a significant level of survival marks the end of the TSP. For adult morphology defects, mutant and normal phenotypes are monitored.

A detailed analysis of the timing and types of defects produced by each mutation in the imaginal disc derivatives are performed by subjecting larvae of known age to specific temperature pulses. Each strain is subjected to 12-hr and 24-hr pulses. Besides these two pulses, each stock is subjected to another 3 × 48 hr-pulse treatment (0 – 48, 48 – 96 and 96 – 144 hrs). The emerging adults are saved and examined for repeats and/or deficiencies of the imaginal disc derivatives. In many instances, the animals that have died as pharate adults have to be dissected out of pupal case.

In addition, the temperature threshold of dl^{ts} mutations is determined by adjusting temperature from 25℃ to 31℃ at 2℃ intervals.

For examination under a scanning electron microscope, live adults are anesthetized and mounted on chucks with silver paint. This examination was carried out in Electron Microscopy Unit of Developmental Biology (JEOL scanning electron microscopes, Japan).

1.3 Studies of genetic mosaics

Using *b*, *gp*, *cd*, *pe* as marker genes, ♀ *b*, *gp*, *cd*, *pe* × ♂ dl^{ts} and ♀ (♀ *b*, *gp*, *cd*, *pe* × ♂ dl^{ts}) × ♂ *b*, *gp*, *cd*, *pe* were performed in homozygous conditions and these experiments provide information on cellular requirements for gene expression, but not on the time of gene expression.

Mosaics are generated by irradiation of larvae produced in crosses of ♀ *b*, *gp*, *cd*, *pe* × ♂ dl^{ts}. Larvae aged 24 to 48 hr at 25℃ are exposed to 1000-R X rays (113 R/min; 150 kvp, 1-min A1 filter) and then shifted to 31℃ for the remainder of development. Because genetically homozygous cells are produced at the time of irradiation, the information on both the timing and the cellar requirements for gene expression has been obtained.

2 RESULTS AND ANALYSES

2.1 Temperature-threshold

The result of the experiment on the determination of temperature threshold is shown in Table 1. At 27℃, the death before emergence does not differ from the control practically. Visible morphological defects are exhibited, for example, the shape of the wing and the size of the body are altered. At 29℃, the bulk of individuals have died at the prepupal stage. Only four flies emerged from pupae. One of these flies suffers wing defect. From the observation of the wing characteristics at 27℃ and 29℃, there is now evidence that the initial mutations have occurred at 27℃ and 29℃, but they are not expressed under standard conditions. At 31℃, almost all animals develop to prepupa, whereas differentiation does not occur at all. Thus, the temperature 29℃ is apparently the threshold for the variability of larvae. On the basis of the above result, it is suitable to set 25℃ as the permissive temperature and 31℃ as the restrictive temperature for the following experiments.

Table 1 Temperature threshold of appearance of dl^{ts} mutations of *Drosophila virilis*

Temperature	Number of flies observed	Death of individuals at stages (%)						Number of flies with mutant wing
		Larval differentiation	Prepupa differentiation	Pupa differentiation			Emergence	
				Initial	Partial	Complete		
25℃	380	0.8 ± 0.14	0	0.3 ± 0.28	0	1.1 ± 0.54	97.8 ± 0.75	0
27℃	379	0	0.8 ± 0.14	1.6 ± 0.64	0	2.1 ± 0.74	96.5 ± 0.94	15
29℃	312	0.6 ± 0.44	86.6 ± 1.92	1.3 ± 1.64	5.1 ± 1.25	5.1 ± 1.25	1.3 ± 0.64	4
31℃	120	12.5 ± 3.02	0	0	0	0	0	0

2.2 Temperature sensitive period for lethality

The TSP and effective LP are inferred from reciprocal shift-up and shift-down experiments. The LP, however, does not necessarily coincide with the actual TSP (Suzuki, 1971). The larval and pupal stages of development have been studied with respect to high temperature and *vice versa* at different successive intervals after these cultures are started.

The larval TSPs for lethality are remarkably similar to those observed by Shellenbarger for *l* (*l*) $N1^{ts}$ (1977). Shift-down experiments have defined the start of the first larval TSP at the second larval instar at 31℃ between 24 and 48 hr after hatching, near the first larval molt. With 24-hr pulses at 31℃ and during the culturing period at 31℃, lethality overlaps either the second larval instar (the first TSP) or the last three-quarters of the third larval instar (the second TSP). A 24-hr-pulse initiated at the second molt (120 hr after hatching) results in nearly normal survival levels and clearly separates these two larval TSPs. Only during the second TSP, 12-hr pulses have produced a convincing decrease in survival. Pulses at the end of the third-instar TSP allow survival and mark the end of the TSP. However, a return to normal survival levels is not observed because of overlap with a pupal TSP.

The LP for animals treated at 31℃ during the second larval instar TSP occurs when they completely have formed imagos inside unbroken pupa cases. When dissected out of the pupal case for histological examination, these animals often have a reduced eye/head size. If treated longer, there is a disorientating of ocellar structures and doubling of antennal parts on the same side as any extreme eye reduction. The LP for animals treated at 31℃ during the third instar TSP occurs in early pupal development and is associated with large black spots of degenerating material in the eye regions. There is possibility of cell degeneration as well as extra growth. Their significance will be discussed in another section.

Pupal TSP begins at approximately the 6-hr point at 31℃ (corresponding to the time of true pupation) and which extends for several hours before complete killing is observed. The end of the TSP is defined by the earliest shift-up which allows survival after 24 to 36 hr at 25℃. The lethal phase for this TSP is at emergence of the fully developed imago.

Pulses of at least 12 hr at 31℃ are required to kill 100% of dl^{ts} pupae while 2-hr and 3-hr pulses are sufficient to kill a few percent of pupae. For each pulse duration, the age for the initiation of the pulse to reach the peak effect is earlier than the TSP defined by shift-up and shift-down experiments. It is conceivable that there is no predictable temporal correlation between TSP an LP. Thus, in some mutants, the two coincide, while in others TSP may precede the LP by an interval of several hours as described by Shellenbarger (1977).

As for the embryonic lethality, with the variation of the temperature maintenance during the embryo stage, no distinct sensitive period is detected, and therefore, the data of these experiments are not presented here.

2.3 TSPs for adult morphology defects

A great deal of eye, wing, leg, and bristle defects produced by 12-hr heat pulses during the larval developmental stage are observed (Table 2).

Table 2 Adult morphology defects of dl^{ts} resulting from 12-hr larval pulses

Age* (h)	Number of adults observed	Number of late pupae	Eye		Wing		Leg		Bristle	
			+	−	+	−	+	−	+	−
0	98	5	95	0	98	0	93	0	93	0
12	89	0	85	0	84	1	83	0	85	0
24	75	3	73	0	73	1	74	0	70	0
36	76	2	73	0	72	0	73	0	74	0
48	75	1	74	0	73	1	72	0	70	0
First molt										
60	72	2	69	0	68	0	69	0	67	0
72	81	0	77	1	78	2	78	0	76	0
84	73	3	70	1	69	1	68	0	68	0
96	101	4	98	0	98	1	99	0	90	0
108	93	1	88	2	4	38	85	0	85	0
Second molt										
120	157	4	112	50	57	98	150	5	156	0
132	159	8	40	112	31	121	119	35	141	0
144	161	10	25	131	27	130	101	56	157	0
156	131	12	0	125	0	123	58	69	125	1
168	121	13	0	117	2	113	48	72	117	0
180	127	10	0	112	5	108	38	86	120	2
192	133	20	1	129	102	21	120	18	125	10
204	89	22	0	85	69	18	70	15	18	65
216	75	25	1	70	59	15	50	17	11	59
228	69	35	5	51	63	2	65	4	12	49
Control										
25℃	59	5	59	0	57	1	58	0	58	0

* : Age at initiation of pulse within hours after hatching. Boxes identify treatments which overlap TSP. (+) Wild type; (−) Mutant.

Eye defects The most distinguished characteristic is roughness usually accompanied by a deep scar, progress from the posterior rim of the eye to the anterior rim as the time of pulse progresses through the third instar. Besides this , the eye defects include: (1) the eyes are small and lozenge-shaped, (2) the colour of the eyes are amber, (3) there is a concentration of pigment around the edge of the eye, (4) there is no facet structure, and some of hairs on the eyes are increased in length, and (5) the eye pigment shows clumbing (Fig. 1).

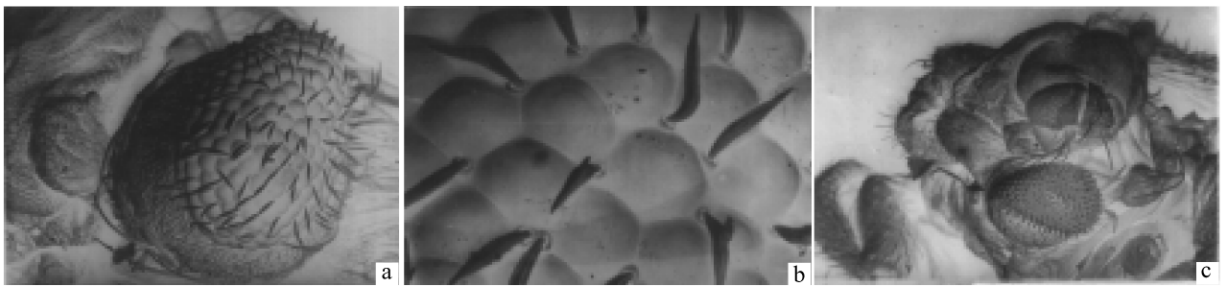

Fig. 1 Scanning electron micrographs of eye defects in dl^{ts} animals pulsed at 31℃ during the third larval instar (a) 12-hr pulse initiated at 156 hr after hatching: eye abnormalities. (b) The details of (a): a cluster of pentagonal ommatidia and occasional misplacements of bristles. (c) 12-hr pulse initiated at 180 hr after hatching: eye scar.

Wing defects Small wing, missing inner and sometimes outer wing margins are produced by pulses in the latter part of the second instar and the third instar respectively. The thick wing vein occurs at the last part of third instar and covers prepupal period near the pupal stage (Fig. 2).

Leg defects Fusion of the femur-tibia joint, fusion of the tibia-tarsal joint and shortened tarsus with missing segments are produced by pulses in the early, middle, and middle to latter part of the TSP respectively. The highest penetrance of all leg defects occur with the metathoracic leg (Fig. 3).

Fig. 2 Scanning election micrographs of thick wing vein, 12-hr-pulse treatment at 31℃, pulse initiated at 228 hr after hatching

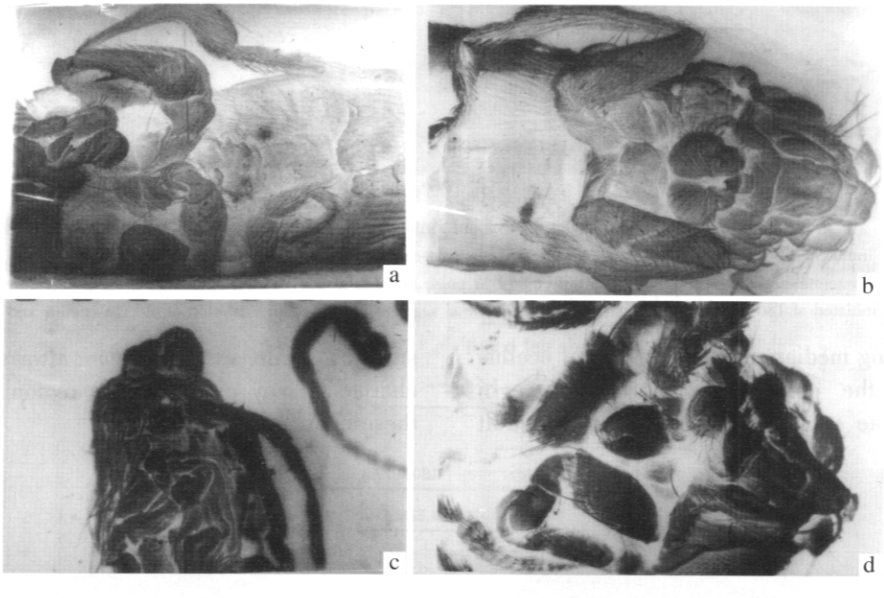

Fig. 3 Scanning electron micrographs showing leg defects in dl^{ts} animals pulsed at 31℃ during the third larval instar
(a) 12-hr pulse initiated at 144 hr after hatching: fused femur-tibia. (b) 12-hr pulse initiated at 156 hr after hatching: shortened tarsus. (c) 12-hr pulse initiated at 168 hr after hatching: fused tibia-tarsal. (d) 12-hr pulse initiated at 180 hr after hatching: shortened femur and tarsus (right leg).

Bristle defects Extra vibrissa, ocellar, postvertical, orbital, sternalpleural and other macrochaetes to a lesser extent are produced by heat pulse in the last part of the third larval instar (Fig. 4). Extra bristles typically arise from separate sockets.

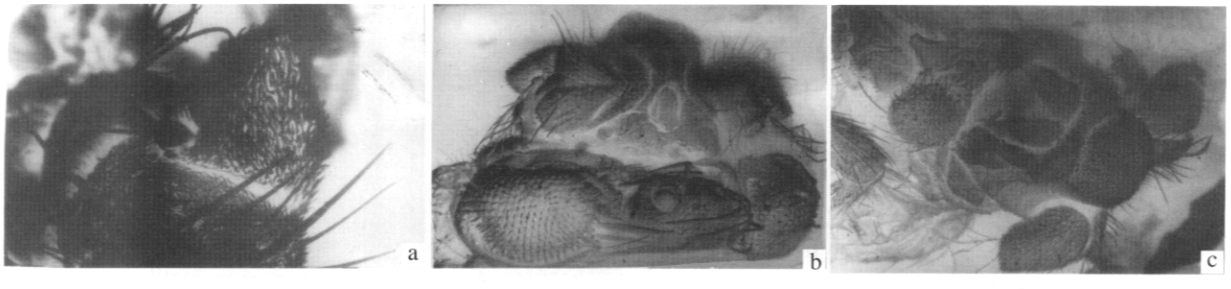

Fig. 4 Scanning electron micrographs of bristle defects in dl^{ts}, animals pulsed at 31℃
(a) 12-hr pulse initiated at 216 hr after hatching: extra bristles arise from the second antennal segment. (b) 12-hr pulse initiated at 192 hr after hatching: cooked bristles and missing lateral ocellus. (c) 12-hr pulse initiated at 228 hr after hatching: missing aristas and bristles, repeat of antennal segment disc derivatives and deletion of eye disc derivative.

Antennal segment defects The antennal segments are usually small with altered shape or absent completely. It is of interest to examine homoetic transformations ranging from the second instar to the end of the third instar (Fig. 5).

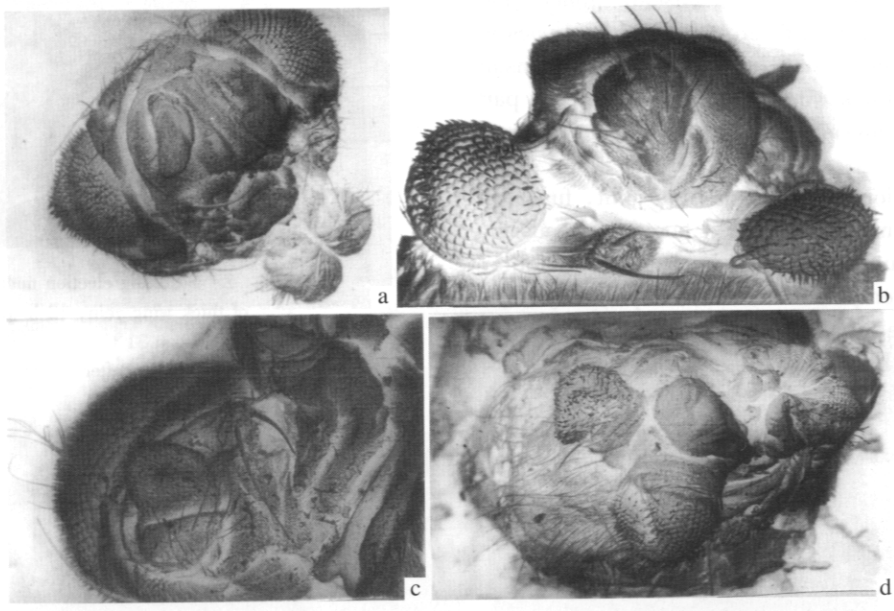

Fig.5 Scanning electron micrographs of antennal defects in dl^{ts}, animals pulsed at 31℃

(a) 12-hr pulse initiated at 108 hr after hatching: one side of antennal segment absent. (b) 12-hr pulse initiated at 144 hr after hatching: extra bristles in the third antennal segment and missing aristle. (c) 12-hr pulse initiated at 204 hr after hatching: missing one side of antennal segments. (d) 12-hr pulse initiated at 180 hr after hatching: deletion of antennal segment derivative and deletion of median ocellus and lateral ocellus.

Extra or missing median ocellus and lateral ocellus have TSP during the end of the third instar. In addition, the chaetae occur over all parts of the adult surface, while extra chaetae always precede missing chartae on any given surface region. Fig. 6 indicates these characteristics.

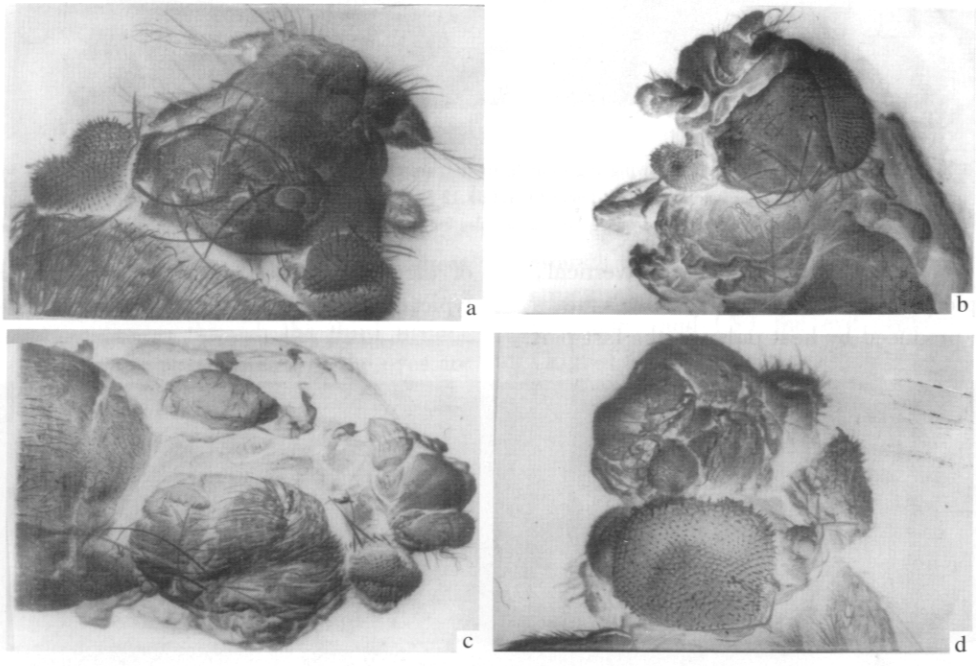

Fig. 6 Scanning electron micrographs of median ocellus and lateral ocellus in dl^{ts}, animals pulsed at 31℃

(a) 12-hr pulse initiated at 228 hr after hatching: repeat of median ocellus. (b) 12-hr pulse initiated at 228 hr after hatching: deletion of median ocellus and lateral ocellus. (c) 12-hr pulse initiated at 216 hr after hatching : deletion of lateral ocellus. (d) 12-hr pulse initiated at 24 hr after hatching: deletion of ocelluses and bristles.

Additional observation is also available here (Fig. 7). With a 12-hr exposure of the second instar larvae at 31℃, the head capsule formation breaks. And with a 16-hr exposure, the head and wing disc do not develop absolutely. Meanwhile, the development of thorax and leg discs is disrupted. After 26-hour exposure,

abdomen disc disappears. The disruption of wing development begins almost simultaneously with the defect of head disc.

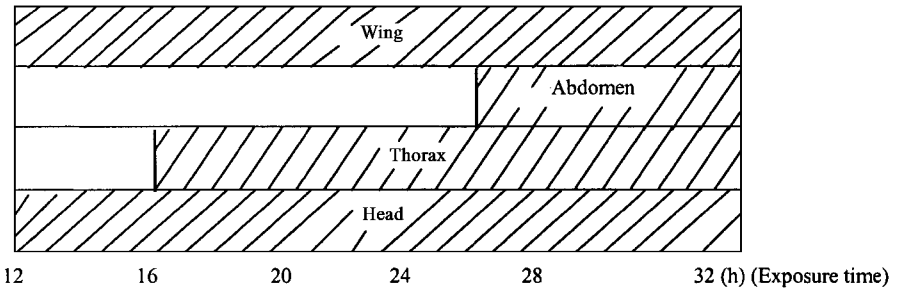

Fig. 7 Developmental abnormalities of the imaginal structure depending on the time of maintenance of 2nd instar dl^{ts} larvae at 31℃
The number in the figure is the exposure time of the larvae. The diagonal part shows the abnormal development of different part.

Drawn from Table 2 and Fig. 7 is the conclusion: By temperature shift experiments, TSP can be defined for each mutant phenotype. This information is useful even in the absence of molecular understanding of a sensitive period. For example, in analyzing temperature-sensitive mutation there is evidence that certain genes may act in different discs at different times during development. So the expression of a mutant phenotype can be experimentally manipulated either by temperature pulses or by continuous development at intermediate temperatures.

A summary of the TSPs of dl^{ts} is present in Fig. 8.

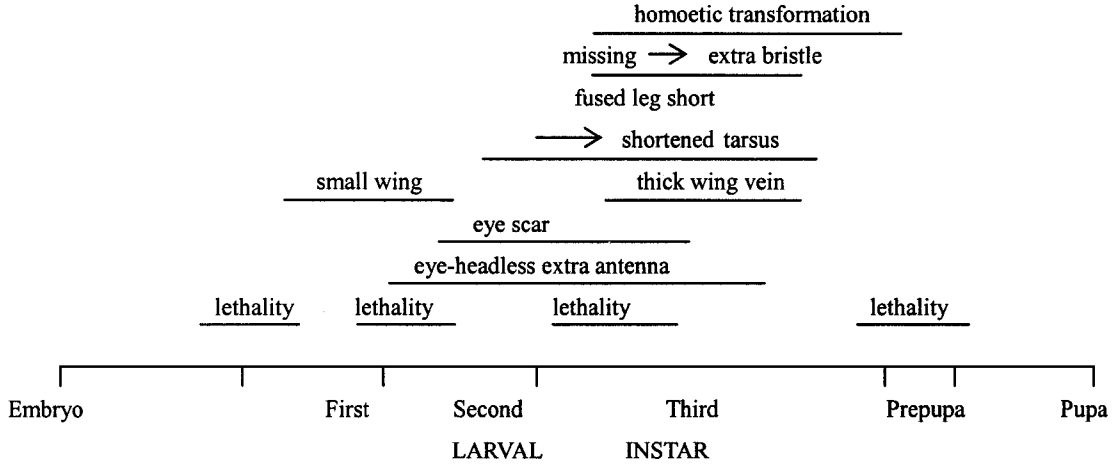

Fig. 8 Summary of the phenotypes and TSPs observed in dl^{ts} homo- and hemizygotes

TSP itself can also be determined by using temperature pulse and by changing the starting points and the duration of the pulses. Additional clues can be found as to the nature of the defects caused by the mutation. For example, if adults recovered from early temperature pulses exhibit wing defects but not leg defects, late pulses give birth to adults with leg defects but not wing defects. It can be concluded that TSP for wing defects is earlier than leg defects. In a word, the availability of several different allele specific temperature sensitive suppressors will be a significant addition to the tools available for analyzing developmental mutants.

2.4 Classification of the mutants

The morphological abnormalities observed in adults which have been subjected to heat pulse during their larval development stage consist of deficiencies and/or repeat of various structures. All of the mutants have been characterized in terms of the patterns of damage demonstrated by the imaginal defects. Each mutant can be put into three different classes: deletion, repeat, and deletion and repeat (Table 3).

Table 3 Correlation between occurrence of different abnormalities with the same imaginal disc derivatives*

Types of abnormalities	Number (observed)	Percent
Deletion only	41	30
Repeat only	5	4
Deletion and Repeat	89	66

* These data are obtained from all the mutations with structural abnormalities in the legs following a 48 – 96-hr temperature pulse.

The extent of the repeats observed in the various defects has ranged from the complete repeat of one structure to the repeat of only one portion of a structure. In general, it has been observed that deficiencies are much more common than repeats. It appears that, within any given structure, deficiencies can occur without repeats, but repeats usually appear to be

associated with deficiencies.

2.5 Phenogenetics of pleiotropic effects

By increasing temperature, the mutations lead to the suppression both of cell division and differentiation of imaginal discs. As a result, the larvae have only developed to prepupal stage. The suppressing effect is also reversible in the first and the second larval instar, but after the second molt, if the larval is subject to such a high temperature, disc cells are no longer capable of dividing, even if the standard conditions are restored. In the second half of first larval instar, although the number of cells in imaginal disc has apparently reached the optimum level of differentiation, the mutations affect the process of differentiation. The temperature can stop the differentiation of many discs, primarily thorax and abdomen discs. The expressive peculiarity of this mutation is the early appearance of cuticle pigment. In general, pigment has already appeared at the beginning of the larvae immobility before pupation. For mutant larvae at 31℃, the pigment that is concentrated on individual spots and is usually around the anal organs is detected in the middle of third instar.

The analysis of phenogenetics offers the promise of understanding the principle that is organized in the imaginal disc development. A survey of the disc affected by such mutations can provide information on which gene functions are required for disc to reveal meaningful pattern.

2.6 Autonomy of dl^{ts} in genetic mosaics

In gynandromorphs for dl^{ts} reared at 31℃, most animals die at late pupae stage. Death is correlated with headless and fused-leg defects: among 61 late pupa gynandromorphs, 23 have only one eye or half a head, 25 have at least one leg fused or missing and only 8 have neither defect, while none of 13 emerged adults has the eye defect, and only 3 have the leg defect. This result for emerged adult and late pupal gynandromorphs combined shows the reduction in expected frequencies of eye and leg parts.

The mitotic recombination experiments show that *pe*. *gp* are absent in these tests, but *cd*, *gp*, *b*, can express well only with *pe* absent. When the generated products from ♀ *b*, *gp*, *cd*, *pe* × ♂ dl^{ts} are subject to heat pulse, only *b*, *gp*, *pe* and *b*, *gp*, *cd* phenotypes are sensitive to temperature.

It is reasonable to believe that dl^{ts} expression is temporally and spatially controlled during the development. TSPs and the autonomy of dl^{ts} indicate that this singe temperature-sensitive function is critically required by all affected structures at given times. Meanwhile, it can not reflect a simple graded series of uniformly depressed activity in all structures. Temperature-shift experiments performed with animals for dl^{ts} and *b*, *gp*, *cd*, *pe* indicate that these mutations have no overlapping temporal defects and this can explain their complementation. Clearly, dl^{ts} cause defects only at a specific time and place. That is to say, that effect is related to some specific-time structures.

3 DISCUSSION

In this study, TSPs and autonomous pleiotropic effects are described for homo- and hemizygotes for the single-site discless, dl^{ts}. For *D*. *virilis*, all of the effects described are known to be dl^{ts} specific function and mapped to a single site between two alleles.

3.1 Adult morphological defects

As to the many aspects of the developmental biology of imagnal discs, the analysis of pleiotropic patterns of damage in lethal mutants was pioneered and inspired by Ernes Hadorn. Comprehensive studies of mutations have been reported. Those studies made extensive inventories of the defects found in a variety of tissues and led Hadorn to the idea that there exist two different kinds of pleiotropy: mosaic and relational. Mosaic pleiotropy refers to a pattern of defects resulting from a mutation in a gene which normally acts autonomously in imaginal cells. This may be accompanied by larval defects. The observations of these experiments on dl^{ts} gene coincide with his description. In our experiments, a great deal of adult defects is observed, but during the larval period, animals seem to develop normally and only histological examination under miocroscope can detect some abnormal discs structures. The precise morphological defects of each disc affected by any given mutation could range from similar to quite different depending on the particular gene product affected and its relative significance for the development of each disc.

The result of these experiments has shown that dl^{ts} gene produces autonomous imaginal disc defects. Such mutants provide an opportunity to examine the effect of the lack of a general function on imaginal disc development. Pulse of restrictive temperature may be applied to such mutants at varying times during the development without resulting in lethality of the treated organisms before metamorphosis. Enclosed or pharate adults may then be examined for defects in imaginal structures. Although a relatively small number of such mutants have been studied in detail, there is a remarkable similarity in their phenotypes. Within each disc, certain structures are missing and others may not be duplicated. For example, in these experiments, the head facets are often missing and the arista and palpus are duplicated.

A unifying hypothesis to account for these results is that imaginal discs respond to localized damage in accordance with their development potential at the time of injury and that whether the injury is internal or

external is largely irrelevant. In fact, Russell (1974) have provided histological evidence that the effect of pulses of restrictive temperature is localized cell death. Such mutants may provide sensitive tools for performing further microsurgery and biochemical analysis.

3.2 Relationship between deletion and repeat

Now, the question is about the mechanism which translates the heat-treated cell death in imaginal discs of a larva into the appearance of deficient, repeat and/or normal structure in the adult.

All adult morphology defects about deletion observed in these experiments can be classified into two groups: one group of pattern-altering mutations deletes very specific pattern element. The second group of pattern-deleting mutations removes large and often variable regions of a disc rather than specially remove individual structures. In same cases this is accompanied by repeat of remaining parts. Some deletion mutation causes all of the discs to be smaller or absent. A lot of defects which have presented in the result section show this characteristic.

The most striking feature of the results discussed so far is the reciprocity between regeneration and repeat; if one disc fragment regenerates, then in almost all of the tested cases the complementary fragment duplicates. Bryant (1974) has advanced a model to illustrate this question, which postulates that a gradient of developmental capacity exists within the disc such that, after the removal of a section of the gradient, the cells at the surface can only produce structures lower on the gradient. The hypothesis provides at least a formal explanation for certain observations. First, the hypothesis implies that heat pulses administered later in development should yield a much higher frequency of deficiencies than those administered early in development. This situation would arise because a disc treated early in development would have more to undergo regeneration than would a disc treated late in development. Conversely, the optimum timing of heat pulses designed to yield duplicated disc derivatives must occur at some earlier developmental stage than those of heat pulses primarily intended to yield deficiencies. The data in Table 3 support this implication of this working hypothesis. A second predication implicit is that genetically mosaic imaginal discs should exhibit repeat of the non-mutant tissue as a result of heat treatment during the larval stage. The result of genetic mosaics can support this prediction. It is implicit in the hypothesis that repeats of an imaginal disc derivative should always be found associated with a deletion for other derivatives of the same imaginal disc. As a matter of fact, many micrographs presented in the result section do correlate with this prediction. It is reasonable to assume that this tissue specificity is the direct result of tissue-specific gene activity.

3.3 Somatic recombination

However, gynandromorphs allow the positive identification of genes which do not act in imaginal discs. Somatic recombination can identify nearly all kinds of genes which do act in imaginal discs. Since recombination only occurs in dividing cells and produces small areas of mutant cells, it can be used as a specific probe of imaginal disc development (Shearn, 1974). Garcia and Merriam (1971) have already provided information on the most useful markers. If the mutation is in a gene which does not act in one or more discs, then no matter when somatic recombination is induced the mutation should have no effect or recovery of marked clones. If the mutation does affect cells in one or more discs autonomously, then the frequency or morphology of marked clones will be significantly altered. First, the precise nature of the alternation depends on the nature of the mutation and when the clone is induced. For example, if the gene only acts early and the clone is induced late, then no effect on recovering will be observed. That is to say, if the gene is only required to function early, then at early times of irradiation mutant clones will express their phenotype. But later, they will look normal. As for the marker strains *b*, *gp*, *cd*, *pe* crossing with dl^{ts}, only *b*, *gp*, *cd*, and *b*, *gp*, *pe* are sensitive to high temperature when offsprings are transferred to 31℃. Probably, this result just comes from the same reason. Second, the phenotype of the marked clone being tested for autonomy depends on the mutation, and it can differentiate abnormally, exhibit a homoetic transformation or even not be recovered at all. pe is absent in such crosses. Moreover, a lot of homoetic transformations are observed from generation individuals. These results are correlated with this postulation, too.

If the holmozygous mutant cell dies, then it will not give rise to any marker cells. If it lives but cannot divide, it will give rise to a patch of one cell, if any at all. If the mutant cell divides but a significantly smaller rate than normal, then the clones found will not only be smaller than control but fewer clones may be found as well. The reduced number might occur because of the difficulty in detecting small clones or because of selection.

However, dl^{ts} acts autonomously and that pleiotropic effects result from independence time-and tissue-specific development requirements. It is very complicated; so far the information in these genetic somatics of dl^{ts} is still scanty. A more meticulous genetic somatic plan is necessary for further analysis of dl^{ts} in *D. virilis*.

Although this work has laid the foundation for a thorough investigation of dl^{ts} in *D. virilis*, many questions still remain to be solved before we understand

molecular mechanism. The possible application of histological and biochemical method of analysis to dl^{ts} gene that causes temperature-sensitive mutations can be made towards understanding the molecular principles of imaginal disc development and perhaps animal development.

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黑果蝇 dl^{ts} 品系的温度敏感时期及 dl^{ts} 基因多效影响的自主性研究

Vladimir G. MITROFANOV[1], 薛　东[2], 杨长举[2], 姚英娟[2], 蔡万伦[2]

(1. Institute of Developmental Biology, Russian Academy of Sciences, Moscow 117334, Russia;
2. 华中农业大学城市有害生物防治研究所, 武汉 430070)

摘要: 本文采用 D.T.Suzuki 的方法, 研究了黑果蝇 *Drosophila virilis* Sturt dl^{ts} 品系的温度敏感时期和致死时期的相互关系。选择了 31℃为限制温度, 25℃为许可温度。对于成虫盘缺损的研究用了 12 个小时为一个脉冲或 24 个小时为一个脉冲的热处理, 用扫描电镜技术辅助对成虫形态缺损的研究。对于成虫盘缺失和重复的关系主要在 48 小时为一个脉冲的热处理盘中进行, 对 dl^{ts} 基因的表达采用了遗传嵌合性的研究, 其结果如下: 1. 两个不连续的温度敏感时态对致死的影响是在第 1 龄幼虫、第 2 龄幼虫、第 3 龄幼虫和进入蛹期后的 10 个小时。温度敏感时态和致死时态并不一致, 而是先于致死时态几个小时。2. 温度敏感时态对成虫形态的影响是: 触角的重复和复眼的缺失发生在第 2 龄和第 3 龄幼虫期。足关节融合及跗节和腿节的缩短发生在第 3 龄幼虫期, 翅脉硬化主要发生在第 3 龄幼虫期即将结束进入前蛹期的这段时间。第 3 龄幼虫期是成虫盘发生缺陷比较集中的时期, 可以明显见到足、复眼、翅和刚毛的缺陷, 同源异型突变体也在这个时期发生。同源突变体的变化主要是足、触角片段及刚毛和触角片段的相互转移。3. 每一个成虫盘缺陷部有自己明显的特征, 根据它们成虫盘的形态缺陷和热处理的时间性, 所有的成虫盘缺陷变化都可以分为这样三类: 缺失, 重复, 缺失和重复并存。4. 遗传镶嵌测试表明: dl^{ts} 基因是自主表达的, 且具有一定的时间、环境和组织的特殊性。

关键词: 黑果蝇; 成虫盘; 温度敏感时态; 致死时态; 模式结构

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